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R-Factors of *Escherichia coli* from Dressed Beef and Humans[1]

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One hundred eighty *Escherichia coli* strains isolated from raw and cooked dressed beef and from healthy humans were screened for resistance to each of nine antibiotics: chlortetracycline, ampicillin, chloramphenicol, kanamycin, neomycin, nalidixic acid, dihydrostreptomycin, oxytetracycline, and tetracycline. Nearly 80% of the 98 beef isolates and 54% of the 82 human isolates were resistant to one or more of the antibiotics tested. Ampicillin resistance was most frequent among beef isolates, and dihydrostreptomycin resistance was most frequent among isolates of human origin. About 74% of the multiply resistant beef strains and 85% of the multiply resistant human strains transferred all or part of their resistances to *E. coli* K-12 recipients.

Transferable drug resistance, mediated by plasmids called R-factors, was first demonstrated in Japan in 1959 (2). Since then, the occurrence of R-factors has been extensively documented and several reviews have been published (3, 5-8, 11). The demonstration that R-factors from *Escherichia coli* isolated from animals could be transferred to resident *E. coli* in humans (10) has lent support to the idea that R-factor-bearing strains of *E. coli* associated with livestock present a potential hazard to humans. Although *E. coli* strains possessing R-factors have been isolated from sausages (9), little is known about the extent to which *E. coli* strains with R-factors are associated with other meat products.

This study (portion of a thesis presented by the senior author in partial fulfillment of the requirements for the M.S. degree in bacteriology at North Dakota State University) was made to determine the incidence of R-factor-bearing strains of *E. coli* in dressed beef. Additionally, the incidence of R-factor-bearing *E. coli* strains in healthy humans was determined for comparative purposes.

MATERIALS AND METHODS

Bacterial strains. Samples of both raw and cooked dressed beef were obtained from the North Dakota State University Food Service, and fecal swabs were provided by students enrolled in bacteriology courses. Presumptive *E. coli* strains were isolated and identified by routine methods; all isolates exhibited typical *E. coli* characteristics on EMB agar, Kligler iron agar, and Simmons citrate agar. Antibiotic-resistant mutants of *E. coli* K-12 F⁻ (ATCC 14948) were employed as recipient strains in all conjugation experiments. Mutagenesis was performed either by ultraviolet irradiation or by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (K and K Laboratories, Plainview, N.Y.) using the procedure described by Adelberg et al. (1). The mutants, designated *E. coli* K-12 C, *E. coli* K-12 K, and *E. coli* K-12 NE, were resistant to 10 μg of chloramphenicol per ml, 50 μg of kanamycin per ml, and 250 μg of nalidixic acid per ml, respectively.

Media and antibiotics. Bacterial cultures were maintained on brain heart infusion (Difco) agar slants and were routinely grown in trypticase soy broth (TSB, Baltimore Biological Laboratories). Penassay (Difco) agar was used as the base in selective media and in the determination of resistances according to the procedure of Bauer et al. (4). The antibiotics contained in the multitipped disks (Consolidated Laboratories, Inc., Glenwood, Ill.), used for the determination of antibiotic-resistance patterns, were ampicillin (10 μg), chloramphenicol (30 μg), chlortetracycline (30 μg), dihydrostreptomycin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), oxytetracycline (30 μg), and tetracycline (30 μg). Ampicillin trihydrate (Parke, Davis, and Co., Detroit, Mich.), chloramphenicol (Sigma Chemical Co., St. Louis, Mo.), kanamycin sulfate (Sigma Chemical Co., St. Louis, Mo.), nalidixic acid (Sigma Chemical Co., St. Louis, Mo.), streptomycin sulfate (Nutritional Biochemicals, Cleveland, Ohio), and tetracycline hydrochloride (The Upjohn Company, Kalamazoo, Mich.) were used in selective media at final concen-

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trations of 10, 10, 30, 100, 10, and 30 μg/ml, respectively.

Conjugation procedure. Conjugation experiments were performed essentially by the procedure described by Watanabe and Fukasawa (12). Each donor or recipient strain was grown in 10 ml of TSB, with shaking, for 6 hr at 37 C. Then, 1-ml portions of the donor and recipient cultures were mixed in a tube containing 2 ml of TSB. After the conjugation mixture had been incubated at 37 C for 18 hr, samples were plated on selective media containing appropriate combinations of antibiotics. Controls consisted of donor and recipient cultures which were incubated and plated separately. When conjugation was achieved, the antibiotic-resistance patterns of representative recombinants were determined with multitipped disks.

RESULTS AND DISCUSSION

A total of 180 strains of *E. coli* was isolated from dressed beef and humans. Although the frequency of isolates having at least one resistance was higher among the 98 dressed beef isolates (79.6%) than among the 82 human isolates (53.7%), resistant human strains averaged considerably more resistances per organism (3.93) than resistant beef strains (2.28). As many as eight resistances were detected in a single dressed beef isolate, whereas seven resistances were the most exhibited by an individual human isolate.

Table 1 presents the frequencies at which resistance to each of the nine antibiotics occurred. Resistance to ampicillin was most common among beef isolates, whereas this resistance was only the fifth most common among human isolates. Resistance to dihydrostreptomycin, the most common resistance for human isolates, was the second most co... among beef strains.

The 11 most frequent of the 21 diffe... resistance patterns exhibited by beef is... are presented in Table 2. Human isolates ... total of 13 different resistance patterns, an... 8 most frequent are shown in Table 3. A ... resistance to ampicillin was, by far, the ... frequent resistance pattern among strains ... dressed beef. Although nonresistant h... isolates were more frequent than human ... lates having a particular resistance patte... higher percentage of resistant human st... were multiply resistant (88.6%) than were ... sistant beef strains (53.7%). Resistance ... chlortetracycline, oxytetracycline, and tetra... cline were usually concurrent.

All resistant isolates were tested for t... ability to transfer their resistances to appr... ate *E. coli* K-12 F⁻ recipients. In insta... where no transfer was initially achieved, p... bly because the selected marker was chrom... mal, or when all resistances were not tr... ferred, the experiment was repeated us...

TABLE 1. *Frequencies of individual resistances*

Resistance*	Beef isolates		Human isolates	
	No. of strains resistant	Percent frequency	No. of strains resistant	Percent frequency
A	14	7.9	34	19.7
AM	60	33.7	18	10.4
C	7	3.9	1	0.6
K	9	5.1	5	2.9
N	7	3.9	5	2.9
NE	8	4.5	3	1.7
S	42	23.6	39	22.5
T	17	9.6	34	19.7
TE	14	7.9	34	19.7

* Abbreviations: A, chlortetracycline; AM, ampicillin; C, chloramphenicol; K, kanamycin; N, neomycin; NE, nalidixic acid; S, dihydrostreptomycin; T, oxytetracycline; TE, tetracycline.

TABLE 2. *Eleven most frequent resistance patte... of beef isolates*

Resistance pattern*	No. of strains	Perc... frequ...
AM	29	29.6
None	20	20.4
AM-S	14	14.3
S	7	7.1
A-AM-K-N-S-T-TE	5	5.1
AM-C-S	3	3.1
NE-S	2	2.0
A-T-TE	2	2.0
NE-S-T	2	2.0
A-S-T-TE	2	2.0
A-AM-T-TE	2	2.0

* For abbreviations, see footnote to Table 1.

TABLE 3. *Eight most frequent resistance patte... of human isolates*

Resistance pattern*	No. of strains	Perc... frequ...
None	38	46.3
A-S-T-TE	16	19.5
A-AM-S-T-TE	8	9.8
AM-S	4	4.9
S	3	3.7
A-T-TE	3	3.7
A-AM-K-N-S-T-TE	3	3.7
AM	2	2.4

* For abbreviations, see footnote to Table 1.

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TABLE 4. *Frequencies of t... resistanc...*

	Beef isolates	
	No. having resistance	No. transferring resistance (%)
	14	7 (50.0)
AM	60	19 (31.7)
	7	3 (42.9)
	9	6 (66.7)
	7	5 (71.4)
N	8	3 (37.5)
	42	36 (85.7)
	17	8 (47.1)
TE	14	7 (50.0)

...e abbreviations, see foot...

...ent recipient strain ... both. By so doing ... we transfer was det... the frequencies at w... determinants we ... transferable at high fr... ted that the conjugal t... alidixic acid has not b...

...idering only multipl... those from dresse... from humans were al... their resistances. ... the most common ... "ampicillin-dih... transferred the resi... transferred only ...reptomycin. The most ... of human isolates. ...streptomycin-oxyte... was completely tran... ates having that patt... study has demons... transferable antibi...

TABLE 4. *Frequencies of transfer for individual resistances*

[illegible]*	Beef isolates		Human isolates	
	No. having resistance	No. transferring resistance (%)	No. having resistance	No. transferring resistance (%)
A	14	7 (50.0)	34	28 (82.4)
AM	60	19 (31.7)	18	10 (55.6)
C	7	3 (42.9)	1	1 (100.0)
K	9	6 (66.7)	5	5 (100.0)
N	7	5 (71.4)	5	5 (100.0)
NE	8	3 (37.5)	3	2 (66.7)
S	42	36 (85.7)	39	35 (89.7)
T	17	8 (47.1)	34	28 (82.4)
TE	14	7 (50.0)	34	28 (82.4)

*For abbreviations, see footnote to Table 1.

ferent recipient strain or selective conditions, or both. By so doing, considerably more resistance transfer was detected. Table 4 indicates the frequencies at which individual resistance determinants were transferred; all were transferable at high frequencies. It should be noted that the conjugal transfer of resistance to nalidixic acid has not been reported previously.

Considering only multiply resistant isolates, 71.8% of those from dressed beef and 84.6% of those from humans were able to transfer all or part of their resistances. Of the beef strains having the most common multiple resistance pattern, "ampicillin-dihydrostreptomycin," 42.9% transferred the resistances completely and 50.0% transferred only resistance to dihydrostreptomycin. The most common resistance pattern of human isolates, "chlortetracycline-dihydrostreptomycin-oxytetracycline-tetracycline," was completely transferred by 93.8% of the isolates having that pattern.

This study has demonstrated a high incidence of transferable antibiotic resistance in *E. coli* strains isolated from dressed beef and healthy humans. If ingested, the R-factor-bearing strains of *E. coli* associated with dressed beef could give rise to serious health problems. However, the *E. coli* strains already present in the humans sampled would seem to be even more potentially harmful because these strains tended to be more multiply resistant and also were capable of transferring their resistances at higher frequencies than could the beef strains.

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